Effect of heating oil on the activity of soil enzymes and the yield of yellow lupine

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ABSTRACT

The aim of the study was to determine the response of soil enzymes such as dehydrogenases, urease and acid and alkaline phosphatases to heating oil contaminating (0.0, 0.25, 0.5, 0.75, 1.0, 1.5% of soil) the experimental soil supplemented with lime and used for cultivation of yellow lupine of the Markiz variety. An increasing contamination of soil with heating oil stimulated the activity of dehydrogenases and acid and alkaline phosphatases but had a toxic effect on yellow lupine. Lime supplements did not have a significant effect on an average activity of soil dehydrogenases. However, such soil treatment had a significant effect on urease. Increasing heating oil doses in lime-supplemented soil stimulated urease activity, whereas in lime-free soil urease activity was inhibited. The activity of acid and alkaline phosphatase was lower in limed soil than in lime-free soil. The activity of dehydrogenases, urease and alkaline phosphatase in the soil with lupine cultivation was significantly higher than in the unsown soil.

Keywords: heating oil; soil contamination; soil enzymes; yield of yellow lupine

Organic and mineral substances, either native or added to the soil as organic matter or contaminants, are subjected to a biotransformation resulting in a modification or a degradation of their chemical structure. The biotransformation is carried out mainly by soil microflora producing a wide variety of soil enzymes that determine the course and direction of metabolic changes.

Petroleum-derived contaminants change the soil structure by decreasing its porosity and damaging its aggregates (Caravaca and Roldán 2003). They also have a negative effect on soil physico-chemical properties and the content of oxygen and humidity in the soil, which disturbs the biological equilibrium and deteriorates plant cultivation conditions (Suominen et al. 2000). They can also significantly modify the soil enzymatic activity (Wyszkowska and Kucharski 2000).

Soil supplementation with lime not only increases its porosity and stability of its aggregates (Stenberg et al. 2000), but also improves its physico-chemical properties (Caravaca and Roldán 2003). This creates more optimum conditions for the development of soil microorganisms.

All of these factors create more optimum conditions for the degradation of hydrocarbons contaminating the soil. Many authors have indicated a positive effect of plants on the microbiological degradation of petroleum hydrocarbons in the soil. Plants provide organic substrates such as carbohydrates and amino acids (Radwan et al. 2000) to microorganisms taking part in the microbiological degradation of petroleum-derived substances. Moreover, they produce and excrete their own oxidoreductases that play an active role in the degradation of petroleum-derived contaminants and prevent contaminants from penetrating the soil profile (Cunningham and Ow 1996).

The aim of the study was to determine the response of soil enzymes such as dehydrogenases, urease and acid and alkaline phosphatases to increasing doses of heating oil contaminating the experimental soil supplemented with lime and used for cultivation of yellow lupine.

MATERIAL AND METHODS

The experiment was carried out in a vegetation hall in four replications on Eutric cambisols [pH_{KCl} 5.6, base saturation (BS) 73.97 mmol(+)/kg and cation exchange capacity (CEC) 53.40 mmol(+)/kg]. The experiment was carried out in spring (April, May and June) and lasted 72 days. Prior to sowing, the

soil in all the pots was fertilised with the same doses of macroelements (expressed as the content of pure chemical element used in the mineral fertiliser): N 0.20 g/kg of soil [$CO(NH_2)_2$], P 0.10 g/kg of soil (KH_2PO_4), K 0.15 g/kg of soil (KH_2PO_4 + KCl), Mg 0.05 g/kg of soil ($MgSO_4.7\ H_2O$) and microelements: Zn 5.0 mg/kg of soil ($ZnCl_2$), Cu 5.0 mg/kg of soil ($CuSO_4.5\ H_2O$), Mn 5.0 mg/kg of soil ($MnCl_2.5\ H_2O$), Mo 5.0 mg/kg of soil ($Na_2MoO_4.2\ H_2O$), B 0.33 mg/kg of soil (H_3BO_3).

The experimental soil was contaminated with following doses of heating oil: 0.0, 0.25, 0.5, 0.75, 1.0, 1.5% of dry soil mass. The doses of heating oil were established based on previous studies in the Department of Microbiology (Wyszkowska and Kucharski 2000). Besides, CaO was added at the amount of 0.39 g/kg of soil to the pots where the cushioning effect of lime on oil-derived contamination was examined. This dose compensated for the entire content of hydrogen ions.

Thoroughly blended soil samples were transferred into polyethylene pots, 3.2 kg per pot. Throughout the entire experimental period (72 days), soil humidity was maintained at 60% of capillary water capacity. The pots were weighed several times a day and watered with distilled water to obtain a fixed weight.

On day 14, half of the pots with limed soil and half of the pots with lime-free soil were sown with yellow lupine variety Markiz seeds (5 seeds per pot) that were treated with Nitragine solution (microbiological inoculant containing *Bradyrhizobium* spp. bacteria) using 2.5 ml per seed, prepared from 1 pack of inoculant and 1 dm^3 of water. The same amount of Nitragine was also added to the seed-free pots.

The lupine was harvested at the blooming phase (72nd day of experiment) and the yield of above-ground parts and roots, as well as mass and the number of nodules were determined. Soil samples for analyses were collected on the day of sowing (14th day) and on the final experimental day, i.e. harvest day (72nd day). Soil samples were stored at 4°C for the maximum of 4 days before they were analysed in laboratory.

The activity of dehydrogenases [Deh] was measured with the Lenhard method modified by Casida (Casida et al. 1964). In this reaction, 2,3,5-triphenyl tetrazolium chloride (TTC), used as 3% aquatic solution, is the electron acceptor. This compound is reduced to triphenyl formazan (TPH) during 24-hour soil incubation at 37°C. The colour intensity of triphenyl formazan (TPH) was measured with a spectrophotometer at the wavelength of 485 nm. The activity of dehydrogenases was expressed in $cm^3\ H_2$/kg d.m. of soil/day. The activity of soil urease [Ure] was measured with the Gorin and Ching Chang method (Gorin and Ching Chang 1966). The soil samples were incubated with 10% aquatic solution of urea as a reaction substrate and citrate buffer (pH 6.7) for 24 h at 37°C. $N\text{-}NH_4$ was measured calorimetrically with the use of the Nessler reagent at the wavelength of 410 nm. The urease activity was expressed in mg $N\text{-}NH_4$/kg d.m. of soil/day. The activity of acid phosphatase [Pac] and alkaline phosphatase [Pal] was measured with 0.1M solution of *p*-nitrophenyl phosphate (PNP) as a reaction substrate. The soil sample was incubated with the addition of PNP and universal buffer of pH 6.5 (for acid phosphatase) and pH 11 (for alkaline phosphatase) at 37°C for 1 h. The content of *p*-nitrophenole (PNP) was measured with a spectrophotometer at the wavelength of 410 nm (Tabatabai and Bremner 1969). The activity of acid and alkaline phosphatases was expressed in mmol PNP/kg d.m. of soil/h. Additionally, the content of organic carbon [C_{org}] was determined (Lityński et al. 1976). Based on the soil enzymatic activity and the content of organic carbon, a biochemical index of soil fertility (M_w) was calculated using the following formula (Kucharski 1997).

$$M_w = (Ure/10 + Deh + Pal + Pac)\cdot\%C_{org}$$

The obtained results were statistically analysed with the Duncan test. The coefficients of the correlation between the heating oil dose and the activity of soil enzymes, as well as between the plant yield (over-ground parts, roots, number of nodules and mass of nodules) and the soil biochemical activity were calculated.

RESULTS AND DISCUSSION

The results of the study indicated a positive effect of heating oil on the enzymatic activity of the soil, however, the level of this activity depended on the enzyme and other experimental factors such as liming or yellow lupine cultivation.

The activity of soil dehydrogenases (Table 1) was positively correlated with increasing doses of heating oil contaminating the soil in all the experimental variants, regardless of the lime content in the soil or cultivation of yellow lupine. The cultivation of yellow lupine had a considerably positive effect on the activity of dehydrogenases, while soil liming had a lesser effect.

Table 1. Activity of dehydrogenases ($cm^3 H_2$/kg d.m. of soil/d) in the soil contaminated with heating oil, limed (+Ca), lime-free (–Ca) and sown, unsown with yellow lupine

Heating oil dose in % of soil mass	Soil				Average
	sown with yellow lupine		unsown with yellow lupine		
	+Ca	–Ca	+Ca	–Ca	
0.00	3.58	4.02	2.68	2.37	3.16
0.25	5.81	5.95	4.35	5.04	5.29
0.50	7.64	7.32	5.85	6.21	6.76
0.75	7.28	8.68	6.60	6.14	7.18
1.00	9.24	9.85	8.19	8.25	8.88
1.50	10.56	10.00	8.56	6.20	8.83
Average	7.35	7.64	6.04	5.70	
r	0.97**	0.98**	0.98**	0.79**	0.87**
LSD	a = 0.11**, b = 0.07**, c = 0.07**, a × b = 0.18**, a × c = 0.18**, b × c = 0.19**, a × b × c = 0.27**				

r = correlation coefficient between dose of heating oil and activity of dehydrogenases, *LSD* = least significant differences, a = heating oil dose, b = sown soil, c = lime application, a × b, a × c, b × c, a × b × c = factor interaction, * and ** – statistically significant differences for $P < 0.05$ and $P < 0.01$, respectively, ns = non significant

The urease response to soil contamination with heating oil depended on its dose and the presence of lime in the soil (Table 2). In lime-free soils, heating oil inhibited the activity of this enzyme (with the strongest inhibition in the soil contaminated with 1.5% dose of heating oil), while in the limed soils the presence of this contaminant clearly stimulated its activity. Cultivation of yellow lupine had a smaller effect, however, urease activity was clearly lower in the lime-free soils uncultivated with yellow lupine.

As in the case with dehydrogenases, heating oil had a positive effect on the activity of soil phosphatases: alkaline phosphatase and acid phosphatase (Tables 3 and 4). However, in both cases, a positive correlation was observed; heating oil had a stronger effect on the activity of dehydrogenases than on the activity of phosphatases. This can

Table 2. Activity of urease (mg $N\text{-}NH_4^+$/kg d.m. of soil/d) in the soil contaminated with heating oil, limed (+Ca), lime-free (–Ca) and sown, unsown with yellow lupine

Heating oil dose in % of soil mass	Soil				Average
	sown with yellow lupine		unsown with yellow lupine		
	+Ca	–Ca	+Ca	–Ca	
0.00	9.31	12.43	9.05	8.32	9.78
0.25	9.04	10.45	7.49	8.31	8.82
0.50	9.99	10.54	9.54	8.36	9.61
0.75	9.48	10.84	9.84	7.18	9.33
1.00	13.84	10.98	11.40	7.75	10.99
1.50	13.41	6.72	13.66	6.91	10.17
Average	10.85	10.33	10.16	7.81	
r	0.79**	–0.71**	0.83**	–0.59*	0.19
LSD	a = 0.37**, b = 0.29**, c = 0.29**, a × b = 0.45**, a × c = 0.45**, b × c = 0.71**, a × b × c = 0.81**				

Explanation see Table 1

Table 3. Activity of acid phosphatase (mmol PNP/kg d.m. of soil/h) in the soil contaminated with heating oil, limed (+Ca), lime-free (–Ca) and sown, unsown with yellow lupine

Heating oil dose in % of soil mass	Soil				Average
	sown with yellow lupine		unsown with yellow lupine		
	+Ca	–Ca	+Ca	–Ca	
0.00	0.79	0.92	0.88	1.02	0.90
0.25	0.88	0.94	0.88	1.02	0.93
0.50	0.90	0.99	0.89	1.04	0.95
0.75	0.90	0.99	0.96	1.11	0.99
1.00	0.95	1.02	0.98	1.20	1.04
1.50	0.97	1.09	1.03	1.26	1.09
Average	0.90	0.99	0.93	1.11	
r	0.95**	0.96**	0.96**	0.96**	0.61**
LSD	a = 0.02**, b = 0.01**, c = 0.01**, a × b = 0.04**, a × c = 0.04**, b × c = 0.02**, a × b × c = 0.04**				

Explanation see Table 1

be explained by the significant representation of oxidoreductases in the microbial degradation of petroleum-derived substances (Tsao et al. 1998). Soil liming had a clearly positive effect on the activity of acid phosphatase, which may be related to the great susceptibility of this enzyme to soil reaction. Acosta-Martinez and Tabatabai (2000), indicate that acidic phosphatase activity dominates in soils with low pH, while alkaline phosphatase dominates in soils with high pH.

Studies completed by other authors (Wyszkowska and Kucharski 2000, Kucharski and Wyszkowska 2001) indicated that the effect of petroleum-derived substances on the activity of soil enzymes depends mainly on the type of the experimental substance (chemical composition of the tested substance) and the experimental conditions and duration. In the study by Wyszkowska and Kucharski (2000), lead and lead-free gasoline inhibited the activity of dehydrogenases, urease and acid and alkaline

Table 4. Activity of alkaline phosphatase (mmol PNP/kg d.m. of soil/h) in the soil contaminated with heating oil, limed (+Ca), lime-free (–Ca) and sown, unsown with yellow lupine

Heating oil dose in % of soil mass	Soil				Average
	sown with yellow lupine		unsown with yellow lupine		
	+Ca	–Ca	+Ca	–Ca	
0.00	0.53	0.54	0.59	0.48	0.54
0.25	0.65	0.62	0.64	0.59	0.63
0.50	0.68	0.77	0.65	0.68	0.69
0.75	0.72	0.83	0.71	0.74	0.75
1.00	0.72	0.84	0.82	0.74	0.78
1.50	0.88	0.89	0.85	0.85	0.87
Average	0.70	0.75	0.71	0.68	0.71
r	0.94**	0.96**	0.96**	0.97**	0.93**
LSD	a = 0.03**, b = 0.02**, c = 0.02**, a × b = ns, a × c = ns, b × c = 0.03**, a × b × c = 0.06**				

Explanation see Table 1

Table 5. Biochemical index of soil fertility (M_w) of soil contaminated with heating oil, limed (+Ca), lime-free (–Ca) and sown, unsown with yellow lupine

Heating oil dose in % of soil mass	Soil				Average
	sown with yellow lupine		unsown with yellow lupine		
	+Ca	–Ca	+Ca	–Ca	
0.00	3.32	4.34	2.73	2.93	3.33
0.25	5.35	6.00	4.15	5.05	5.14
0.50	7.02	7.07	6.00	6.35	6.61
0.75	8.05	9.47	7.67	7.44	8.16
1.00	10.80	11.19	9.98	9.50	10.37
1.50	13.11	12.11	11.47	8.57	11.31
Average	7.94	8.36	7.00	6.64	
r	0.99**	0.99**	0.99**	0.95**	0.95**
LSD	a = 0.17**, b = 0.10**, c = 0.10**, a × b = 0.24**, a × c = 0.24**, b × c = 0.26**, a × b × c = 0.34**				

Explanation see Table 1

phosphatases. In other studies by Kucharski and Wyszkowska (2001), a stimulating effect of diesel oil on the activity of dehydrogenases, urease and phosphatases in unsown soil was observed.

The positive effect of lupine cultivation on the activity of soil dehydrogenases, as well as on the enhanced degradation of contamination observed in the experiment was confirmed in the literature.

Table 6. Yield of yellow lupine and mass and number of root nodules grown in unlimed (–Ca) and limed (+Ca) soils differently treated with heating oil

Heating oil dose in % of soil mass	Yield d.m. in g/pot						Number of nodules per pot	
	above-ground parts		roots		nodules			
	+Ca	–Ca	+Ca	–Ca	+Ca	–Ca	+Ca	–Ca
0.00	16.35	13.79	13.21	3.29	4.94	5.98	50.75	68.75
0.25	1.95	1.97	0.94	0.78	0.08	0.00	0.25	0.00
0.50	1.76	0.98	0.55	0.22	0.00	0.00	0.00	0.00
0,75	1.11	1.12	0.20	0.41	0.00	0.00	0.00	0.00
1.00	0.90	0.88	0.24	0.40	0.00	0.00	0.00	0.00
1.50	0.59	0.81	0.14	0.16	0.00	0.00	0.00	0.00
Average	3.78	3.26	2.55	0.88	0.84	1.00	8.50	11.40
r	–0.67	–0.65	–0.64	–0.69	–0.61	–0.60	–0.61	–0.60
LSD								
a	0.97**		0.18**		0.49**		8.16**	
b	0.68**		0.13**		0.28**		4.99*	
a × b	1.66**		0.31**		0.70**		12.21**	

r = correlation coefficient between dose of heating oil and yield of yellow lupine and mass and number of root nodules, *LSD* = least significant differences, a = heating oil dose, b = lime application, a × b = factor interaction, * and ** – statistically significant differences for $P < 0.05$ and $P < 0.01$, respectively

Table 7. Correlation coefficients between variable factors in the experiment

Variable	Yield		Number of nodules	Mass of nodules	Deh	Ure	Pal	Pac
	above-ground parts	roots						
Yield of roots	0.88*	–	–	–	–	–	–	–
Number of nodules	0.98*	0.76*	–	–	–	–	–	–
Mass of nodules	0.96*	0.72*	0.97*	–	–	–	–	–
Deh	–0.74*	–0.61*	–0.71*	–0.70*	–	–	–	–
Ure	–0.04	–0.09	0.01	0.02	0.39	–	–	–
Pal	–0.71*	–0.54*	–0.69*	–0.68*	0.88*	0.19	–	–
Pac	–0.42*	–0.46*	–0.33	–0.31	0.34	–0.34	0.52*	–
M_w	–0.68*	–0.58*	–0.63*	–0.62*	0.96*	0.39	0.92*	0.46*

*statistically significant differences for $P < 0.05$

Deh = dehydrogenase, Ure = urease, Pal = alkaline phosphatase, Pac = acid phosphatase, M_w = biochemical index of soil fertility

The positive effect of triticale cultivation on the activity of dehydrogenases, urease and acid and alkaline phosphatases in soil free from petroleum-derived contaminants and contaminated with soil (2 cm^3/kg soil) was observed by Wyszkowska and Kucharski (2000). Also, Cunningham and Ow (1996) report the utilisation of papilionaceous plants in phytoremediation of soil contaminated with petroleum-derived hydrocarbons. Root secretions of *Papilionaceae* plants are rich in nitrogen necessary in the microbial degradation of hydrocarbons. Thus, they are similar to grass with an expanded, thick root system to stimulate the biochemical transformations of hydrocarbons.

The biochemical index of soil fertility was calculated based on the activity of the soil experimental enzymes and the content of organic carbon (Table 5). Its mean value was significantly correlated with soil contamination with heating oil regardless of such factors as soil liming or yellow lupine cultivation. Soil liming did not have a considerable effect on the value of the index. However, the value of M_w was significantly higher in the soil cultivated with yellow lupine than in the unsown soil contaminated with heating oil.

Apart from the positive effect of heating oil on the biochemical index of soil fertility, the yield of the above-ground parts and roots of yellow lupine decreased with increasing doses of soil contamination both in limed and lime-free soils with an average yield of the above-ground parts and roots being greater in the limed soil (Table 6). Heating oil present in the soil inhibited the development of root nodules. Even the lowest dose of oil caused a complete atrophy of nodules in yellow lupine cultivated in lime-free soil. The lack of nodules in yellow lupine cultivated in the limed soil was observed at the contaminant doses of 0.5% and higher. In spite of the effective use of plants in the soil bioremediation, hydrocarbons have a negative effect disturbing seed germination and seedling development (mainly determined by plant systemic participation) (Adam and Duncan 2002), as well as lowering plant yield (Wyszkowska and Kucharski 2000).

The yields of the above-ground parts and roots of yellow lupine were negatively correlated with the activity of dehydrogenases and acid and alkaline phosphatases as well as with the biochemical index of soil fertility (Table 7). However, under normal conditions in uncontaminated soil, this correlation is positive.

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